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Specification and Drawing, as originally filed, with Application for Patent Serial No: **2,398,765**, on August 19, 2002, by **LORUS THERAPEUTICS INC.**, assignee of Raed Al-Qawasmeh and Aiping Young, for "2.4.5-Trisubstituted Imidazoles and Their Uses as Anti-Microbial Agents ".

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Sylvie Gregoire
Agent certificateur/Certifying Officer

September 12, 2003
Date

Canada

(CIPO 68)
04-09-02

OPIC CIPO

2,4,5-TRISUBSTITUTED IMIDAZOLES AND THEIR USE AS ANTI-MICROBIAL AGENTS

ABSTRACT

The present invention provides therapeutically effective 2,4,5-trisubstituted imidazole compounds, methods of preparing the same, and pharmaceutical compositions comprising the compounds alone or in combination with other agents. The present invention further provides for the use of the compounds as anti-microbial agents. The anti-microbial properties of the compounds include anti-bacterial and/or anti-fungal activity.

FIELD OF THE INVENTION

This invention pertains to the field of anti-microbial compounds and, in particular, to the use of 2,4,5-trisubstituted imidazole compounds in the treatment of microbial infections.

BACKGROUND OF THE INVENTION

There is currently an urgent need for compounds with broad-spectrum anti-microbial activity for the preparation of new anti-microbial agents. The increasing incidence of infectious disease caused by microbial pathogens in both communities and hospitals is a worldwide health concern. Severe invasive infections are reported as the main complication in cancer therapies, as well as bone marrow transplantation and major surgeries. Infection is also a major concern for immuno-compromised patients with haematological malignancy and/or AIDS.

Amongst bacterial pathogens, there has recently been a significant increase of multi-drug resistance. For example, strains of *Staphylococcus aureus* (methicillin-resistant or MRSA) and coagulase-negative Staphylococci (CoNS) have become resistant to the most commonly used antibiotics, such that the only available antibiotics uniformly active against them are the glycopeptides, vancomycin and teicoplanin. *S. aureus* is one of the leading causes of hospital-acquired bacteremia capable of causing a wide range of diseases ranging from superficial skin infections to potentially fatal illnesses such as bloodstream infection, endocarditis and pneumonia (Diekema *et al. Clin. Infect. Dis.* 2001, 32:S114-132). Other human pathogens that have begun to develop resistance to multiple antibiotics include *Streptococcus pneumoniae* (the leading cause of nosocomial infections) and *Pseudomonas aeruginosa*, *Haemophilus influenzae* and *Moraxella catarrhalis* (the most common community-acquired respiratory pathogens; Hoban *et al. Clin. Infect. Dis.* 2001, 32:S81-93).

Fungal infections are also becoming a major health concern for a number of reasons, including the limited number of anti-fungal agents currently available, the increasing incidence of species resistant to older anti-fungal agents, and the growing population of immuno-compromised patients at risk for opportunistic fungal infections. The most common clinical fungal isolate is *Candida albicans* (comprising about 19% of all isolates). In one study, nearly 40% of all deaths from hospital-acquired infections were due to fungi (Sternberg, *Science*, 1994, 266:1632-1634).

Thus, new classes of anti-microbial agents are needed to address both the growing resistance amongst microbes to present therapies and the general lack of efficacy of existing antibiotics against slow-growing organisms.

Heterocyclic compounds, especially heterocyclic azole derivatives, have been shown to have a wide spectrum of biological activities. One class of compounds with interesting biological activities is the imidazoles (derivatives containing a five-membered heterocyclic azole). A variety of biological activities have been reported for imidazole derivatives with different substitution patterns (Lee *et al. Nature* 1994 327:739-745; Abdel-Meguid *et al. Biochemistry*, 1994, 33:11671; Heerding *et al. Bioorg. Med. Chem. Lett.* 2001, 11:2061-2065; Bu *et al. Tetrahedron Lett.* 1996, 37:7331-7334; Lewis JR. *Nat. Prod. Rep.* 1999, 16:389-418; Lewis JR. *Nat. Prod. Rep.* 1998, 15:417-437 and 371-395).

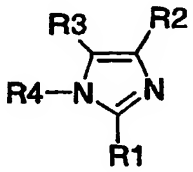
Biological activities have also been reported for aryl-imidazole derivatives, for example, these compounds can act as modulators of multi-drug resistance in cancer cells (Zhang *et al. Bioorg. Med. Chem. Lett.* 2000, 10:2603-2605), inhibitors of p38 MAP kinase (Adams *et al. Bioorg. Med. Chem. Lett.* 2001, 11:867-2870, McLay *et. al. Bioorg. Med. Chem.* 2001, 9:537-554) and of cytokines (U.S. Patent Nos. 5,656,644; 5,686,455; 5,916,891; 5,945,418; and 6,268,370), and inhibitors of bacterial growth (Antolini *et al. Bioorg. Med. Chem. Lett.* 1999, 9:1023-1028).

Recent reports have indicated that triaryl-imidazole compounds can act as inhibitors of p38 MAP kinase (for example, see LoGrasso *et al. Biochemistry*. 1997, 36:10422-10427) and as

modulators of multi-drug resistance in cancer cells (Sarshar *et al. Bioorg. Med. Chem. Lett.* 2000, 10:2599-2601), however, these compounds have found use mainly as colour producing reagents (U.S. Patent Nos. 4,089,747; 5,024,935; 5,047,318; 5,496,702; 5,514,550; and 5,693,589) and as photopolymerization initiators (U.S. Patent Nos. 6,117,609 and 6,060,216), generally in dimeric form.

SUMMARY OF THE INVENTION

It is an object of the present invention to provide a class of compounds which are 2,4,5-trisubstituted imidazole derivatives that have anti-microbial activity. In one aspect of the present invention there is provided a compound of Formula I, and stereoisomers thereof:



(I)

wherein:

R1, R2 and R3 are independently cycloalkyl, substituted cycloalkyl, aryl, substituted aryl, heterocycle, heteroaryl, substituted heterocycle, or substituted heteroaryl;

R4 is hydrogen, halogen, hydroxyl, thiol, lower alkyl, substituted lower alkyl, alkenyl, alkenyl, alkylalkenyl, alkyl alkynyl, alkoxy, alkylthio, acyl, aryloxy, amino, amido, carboxyl, aryl, substituted aryl, heterocycle, heteroaryl, substituted heterocycle, heteroalkyl, cycloalkyl, substituted cycloalkyl, alkylcycloalkyl, alkylcycloheteroalkyl, nitro, or cyano.

In another aspect of the present invention, there is provided the use of a compound of Formula I in the inhibition of bacterial or fungal growth.

In another aspect of the present invention, there is provided the use of a compound of Formula I in the treatment of a bacterial or fungal infection in a mammal in need of such therapy.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 depicts the bactericidal effect of compounds of Formula I against multi-drug resistant *Staphylococcus aureus* (CMRSA-1B).

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides a class of 2,4,5-trisubstituted imidazole compounds and for their use as anti-microbial agents. In the context of the present invention, the term "anti-microbial" refers to the inhibition, prevention or eradication of the growth or proliferation of bacteria and/or fungi and to the inhibition, prevention or eradication of the growth or proliferation of microbially-infected cells.

Definitions

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention pertains.

The terms are defined as follows:

The term "halogen" refers to fluorine, bromine, chlorine, and iodine atoms.

The term "hydroxyl" refers to the group -OH.

The term "thiol" or "mercapto" refers to the group -SH, and -$S(O)_{0-2}$-.

The term "lower alkyl" refers to a straight chain or branched, or cyclic, alkyl group of one to ten carbon atoms. This term is further exemplified by such groups as methyl, ethyl, *n*-propyl, *i*-propyl, *n*-butyl, t-butyl, l-butyl (or 2-methylpropyl), cyclopropylmethyl, *i*-amyl, *n*-amyl, hexyl and the like.

The term "substituted lower alkyl" refers to lower alkyl as just described including one or more groups such as hydroxyl, thiol, alkylthiol, halogen, alkoxy, amino, amido, carboxyl, cycloalkyl, substituted cycloalkyl, heterocycle, cycloheteroalkyl, substituted cycloheteroalkyl, acyl, carboxyl, aryl, substituted aryl, aryloxy, hetaryl, substituted hetaryl, aralkyl, heteroaralkyl, alkyl alkenyl, alkyl alkynyl, alkyl cycloalkyl, alkyl cycloheteroalkyl, cyano. These groups may be attached to any carbon atom of the lower alkyl moiety.

The term "alkenyl" refers to a group -CR'=CR"R"' where R', R", R"' are each independently selected from hydrogen, halogen, lower alkyl, substituted lower alkyl, acyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl or the like as defined.

The term "alkynyl" refers to a group -C C-R'; where R' is selected from hydrogen, halogen, lower alkyl, substituted lower alkyl, acyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl or the like as defined.

The term "alkyl alkenyl" refers to a group -R-CR'=CR"R"", where R is lower alkyl, or substituted lower alkyl, R', R"', R"" are each independently selected from hydrogen, halogen, lower alkyl, substituted lower alkyl, acyl, aryl, substituted aryl, hetaryl, or substituted hetaryl as defined below.

The term "alkyl alkynyl" refers to a group –R-C CR' where R is lower alkyl or substituted lower alkyl, R' is hydrogen, lower alkyl, substituted lower alkyl, acyl, aryl, substituted aryl, hetaryl, or substituted hetaryl as defined below.

The term "alkoxy" refers to the group -OR, where R is lower alkyl, substituted lower alkyl, acyl, aryl, substituted aryl, aralkyl, substituted aralkyl, heteroalkyl, heteroarylalkyl, cycloalkyl, substituted cycloalkyl, cycloheteroalkyl, or substituted cycloheteroalkyl as defined below.

The term "alkylthio" denotes the group -SR, $-S(O)_{n=1-2}$ -R, where R is lower alkyl, substituted lower alkyl, aryl, substituted aryl aralkyl or substituted aralkyl as defined below.

The term "acyl" refers to groups -C(O)R, where R is hydrogen, lower alkyl substituted lower alkyl, aryl, substituted aryl.

The term "aryloxy" refers to groups -OAr, where Ar is an aryl, substituted aryl, heteroaryl, or substituted heteroaryl group as defined below.

The term "amino" refers to the group NRR', where R and R' may independently be hydrogen, lower alkyl, substituted lower alkyl, aryl, substituted aryl, hetaryl, cycloalkyl, or substituted hetaryl as defined below or acyl.

The term "amido" refers to the group -C(O)NRR', where R and R' may independently be hydrogen, lower alkyl, substituted lower alkyl, aryl, substituted aryl, hetaryl, substituted hetaryl as defined below.

The term "carboxyl" refers to the group -C(O)OR, where R may independently be hydrogen, lower alkyl, substituted lower alkyl, aryl, substituted aryl, hetaryl, substituted hetaryl and the like as defined.

The terms "aryl" or "Ar" refer to an aromatic carbocyclic group having at least one aromatic ring (e.g., phenyl or biphenyl) or multiple condensed rings in which at least one ring is aromatic, (e.g., 1,2,3,4-tetrahydronaphthyl, naphthyl, anthryl, or phenanthryl, 9-fluorenyl etc.).

The term "substituted aryl" refers to aryl optionally substituted with one or more functional groups, e.g., halogen, hydroxyl, thiol, lower alkyl, substituted lower alkyl, trifluoromethyl, alkenyl, alkenyl, alkylalkenyl, alkyl alkynyl, alkoxy, alkylthio, acyl, aryloxy, amino, amido, carboxyl, aryl, substituted aryl, heterocycle, heteroaryl, substituted heterocycle, heteroalkyl, cycloalkyl, substituted cycloalkyl, alkylcycloalkyl, alkylcycloheteroalkyl, nitro, sulfamido or cyano.

The term "heterocycle" refers to a saturated, unsaturated, or aromatic carbocyclic group having a single ring (e.g., morpholino, pyridyl or furyl) or multiple condensed rings (e.g., naphthpyridyl, quinoxalyl, quinolinyl, indolizinyl, indanyl or benzo[b]thienyl) and having at least one hetero atom, such as N, O or S, within the ring.

The term "substituted heterocycle" refers to heterocycle optionally substituted with, halogen, hydroxyl, thiol, lower alkyl, substituted lower alkyl, trifluoromethyl, alkenyl, alkenyl, alkylalkenyl, alkyl alkynyl, alkoxy, alkylthio, acyl, aryloxy, amino, amido, carboxyl, aryl, substituted aryl, heterocycle, heteroaryl, substituted heterocycle, heteroalkyl, cycloalkyl, substituted cycloalkyl, alkylcycloalkyl, alkylcycloheteroalkyl, nitro, sulfamido or cyano and the like.

The terms "heteroaryl" or "hetar" refer to a heterocycle in which at least one heterocyclic ring is aromatic.

The term "substituted heteroaryl" refers to a heterocycle optionally mono or poly substituted with one or more functional groups, e.g., halogen, hydroxyl, thiol, lower alkyl, substituted lower alkyl, trifluoromethyl, alkenyl, alkenyl, alkylalkenyl, alkyl alkynyl, alkoxy, alkylthio, acyl, aryloxy, amino, amido, carboxyl, aryl, substituted aryl, heterocycle, heteroaryl, substituted heterocycle, heteroalkyl, cycloalkyl, substituted cycloalkyl, alkylcycloalkyl, alkylcycloheteroalkyl, nitro, sulfamido or cyano and the like.

The term "aralkyl" refers to the group -R-Ar where Ar is an aryl group and R is lower alkyl or substituted lower alkyl group. Aryl groups can optionally be unsubstituted or substituted

with, e.g., halogen, lower alkyl, alkoxy, alkyl thio, trifluoromethyl, amino, amido, carboxyl, hydroxyl, aryl, aryloxy, heterocycle, hetaryl, substituted hetaryl, nitro, cyano, alkylthio, thiol, sulfamido and the like.

The term "heteroalkyl" refers to the group -R-Het where Het is a heterocycle group and R is a lower alkyl group. Heteroalkyl groups can optionally be unsubstituted or substituted with e.g., halogen, lower alkyl, lower alkoxy, lower alkylthio, trifluoromethyl, amino, amido, carboxyl, hydroxyl, aryl, aryloxy, heterocycle, hetaryl, substituted hetaryl, nitro, cyano, alkylthio, thiol, sulfamido and the like.

The term "heteroarylalkyl" refers to the group -R-HetAr where HetAr is an heteroaryl group and R lower alkyl or substituted loweralkyl. Heteroarylalkyl groups can optionally be unsubstituted or substituted with, e.g., halogen, lower alkyl, substituted lower alkyl, alkoxy, alkylthio, aryl, aryloxy, heterocycle, hetaryl, substituted hetaryl, nitro, cyano, alkylthio, thiol, sulfamido and the like.

The term "cycloalkyl" refers to a cyclic or polycyclic alkyl group containing 3 to 15 carbon. For polycyclic groups, these may be multiple condensed rings in which one of the distal rings may be aromatic (e.g. tetrahydronaphthalene, etc.).

The term "substituted cycloalkyl" refers to a cycloalkyl group comprising one or more substituents with, e.g halogen, hydroxyl, thiol, lower alkyl, substituted lower alkyl, trifluoromethyl, alkenyl, alkenyl, alkylalkenyl, alkyl alkynyl, alkoxy, alkylthio, acyl, aryloxy, amino, amido, carboxyl, aryl, substituted aryl, heterocycle, heteroaryl, substituted heterocycle, heteroalkyl, cycloalkyl, substituted cycloalkyl, alkylcycloalkyl, alkylcycloheteroalkyl, nitro, sulfamido or cyano and the like.

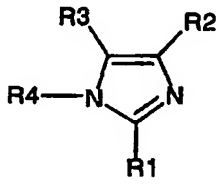
The term "cycloheteroalkyl" refers to a cycloalkyl group wherein one or more of the ring carbon atoms is replaced with a heteroatom (e.g., N, O, S or P).

The term "substituted cycloheteroalkyl" refers to a cycloheteroalkyl group as herein defined which contains one or more substituents, such as halogen, lower alkyl, lower alkoxy, lower alkylthio, trifluoromethyl, amino, amido, carboxyl, hydroxyl, aryl, aryloxy, heterocycle, hetaryl, substituted hetaryl, nitro, cyano, alkylthio, thiol, sulfamido and the like.

The term "alkyl cycloalkyl" refers to the group -R-cycloalkyl where cycloalkyl is a cycloalkyl group and R is a lower alkyl or substituted lower alkyl. Cycloalkyl groups can optionally be unsubstituted or substituted with e.g. halogen, lower alkyl, lower alkoxy, lower alkylthio, trifluoromethyl, amino, amido, carboxyl, hydroxyl, aryl, aryloxy, heterocycle, hetaryl, substituted hetaryl, nitro, cyano, alkylthio, thiol, sulfamido and the like.

I. 2,4,5-Trisubstituted Imidazole Compounds

The present invention provides compounds of the general formula (I):



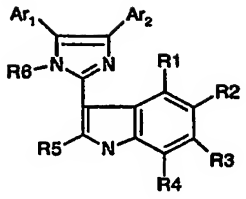
(I)

wherein:

R1, R2 and R3 are independently aryl, substituted aryl, heterocycle, heteroaryl, substituted heterocycle, or substituted heteroaryl;

R4 is hydrogen, halogen, hydroxyl, thiol, lower alkyl, substituted lower alkyl, alkenyl, alkenyl, alkylalkenyl, alkyl alkynyl, alkoxy, alkylthio, acyl, aryloxy, amino, amido, carboxyl, aryl, substituted aryl, heterocycle, heteroaryl, substituted heterocycle, heteroalkyl, cycloalkyl, substituted cycloalkyl, alkylcycloalkyl, alkylcycloheteroalkyl, nitro, or cyano.

In one embodiment of the present invention, the compound of Formula I is selected from:



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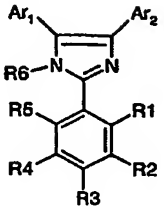

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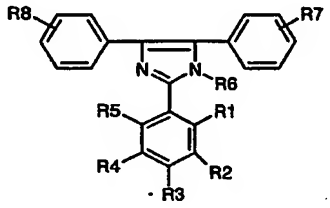
wherein:

Ar_2 and Ar_3 are independently selected from aryl and substituted aryl;

R1, R2, R3, R4, R5 and R6 are indepently selected from hydrogen, halogen, hydroxyl, thiol, lower alkyl, substituted lower alkyl, alkenyl, alkenyl, alkylalkenyl, alkyl alkynyl, alkoxy, alkylthio, acyl, aryloxy, amino, amido, carboxyl, aryl, substituted aryl, heterocycle, heteroaryl, substituted heterocycle, heteroalkyl, cycloalkyl, substituted cycloalkyl, alkylcycloalkyl, alkylcycloheteroalkyl, nitro, or cyano.

In another embodiment of the invention, the compound of Formula I is selected from:



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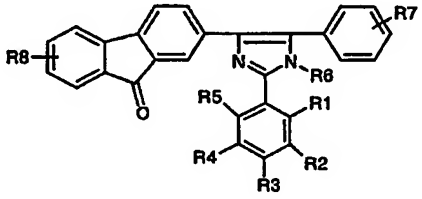

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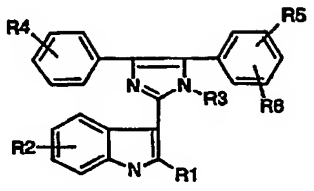

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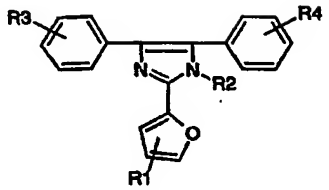

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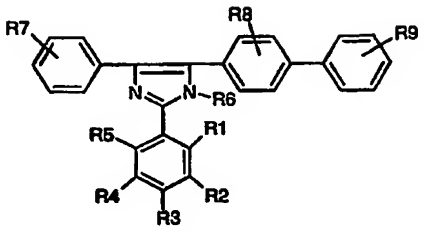
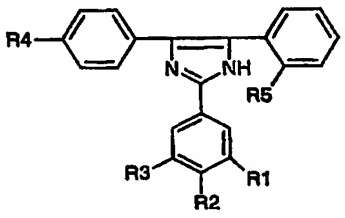


7

wherein:

R1, R2, R3, R4, R5, R6, R7, R8 and R9 are independently selected from hydrogen, halogen, hydroxyl, thiol, lower alkyl, substituted lower alkyl, alkenyl, alkenyl, alkylalkenyl, alkyl alkynyl, alkoxy, alkylthio, acyl, aryloxy, amino, amido, carboxyl, aryl, substituted aryl, heterocycle, heteroaryl, substituted heterocycle, heteroalkyl, cycloalkyl, substituted cycloalkyl, alkylcycloalkyl, alkylcycloheteroalkyl, nitro, or cyano.

In another embodiment of the invention the compound of Formula I is selected from:



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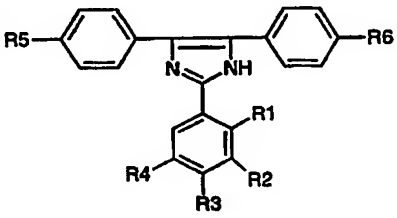

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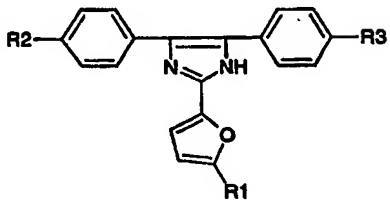

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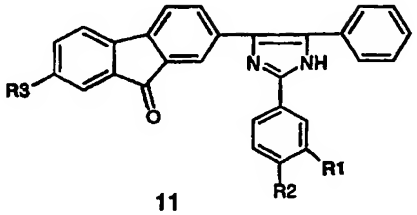

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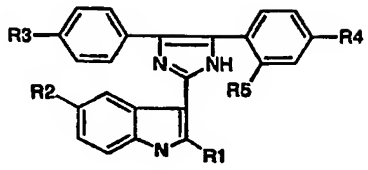

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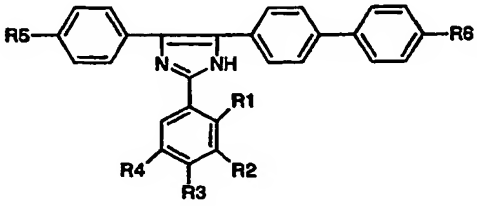

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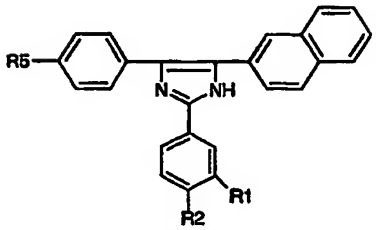
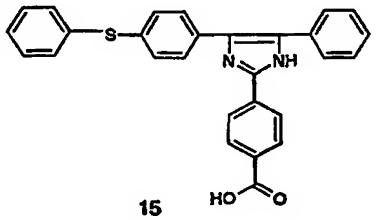


14

wherein:

R1, R2, R3, R4, R5 and R6 are independently selected from hydrogen, halogen, hydroxyl, thiol, lower alkyl, substituted lower alkyl, alkenyl, alkenyl, alkylalkenyl, alkyl alkynyl, alkoxy, alkylthio, acyl, aryloxy, amino, amido, carboxyl, aryl, substituted aryl, heterocycle, heteroaryl, substituted heterocycle, heteroalkyl, cycloalkyl, substituted cycloalkyl, alkylcycloalkyl, alkylcycloheteroalkyl, nitro, or cyano.

In another embodiment of the invention the compound of Formula I is selected from:



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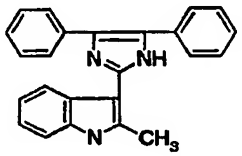

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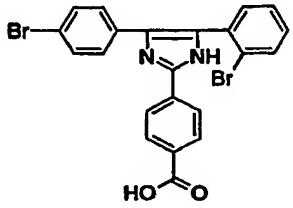

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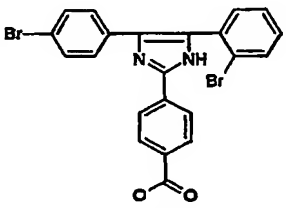

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The present invention includes pharmaceutically acceptable salts of the compounds defined by Formula I. Compounds according to the present invention can possess a sufficiently acidic, a sufficiently basic, or both functional groups, and accordingly react with a number of organic and inorganic bases, and organic and inorganic acids, to form pharmaceutically acceptable salts.

The term "pharmaceutically acceptable salt" as used herein, refers to a salt of a compound of Formula I, which is substantially non-toxic to living organisms. Typical pharmaceutically acceptable salts include those salts prepared by reaction of the compound of the present invention with a pharmaceutically acceptable mineral or organic acid or an organic or inorganic base. Such salts are known as acid addition and base addition salts.

Acids commonly employed to form acid addition salts are inorganic acids such as hydrochloric acid, hydrobromic acid, hydroiodic acid, sulphuric acid, phosphoric acid, and the like, and organic acids such as *p*-toluenesulphonic acid, methanesulphonic acid, oxalic acid, *p*-bromophenylsulphonic acid, carbonic acid, succinic acid, citric acid, benzoic acid, acetic acid, and the like. Examples of such pharmaceutically acceptable salts are the sulphate, pyrosulphate, bisulphate, sulphite, phosphate, monohydrogenphosphate, dihydrogenphosphate, metaphosphate, pyrophosphate, bromide, iodide, acetate, propionate, decanoate, caprylate, acrylate, formate, hydrochloride, dihydrochloride, isobutyrate, caproate, heptanoate, propiolate, oxalate, malonate, succinate, suberate, sebacate, fumarate, maleate, butyne-1,4-dioate, hexyne-1,6-dioate, benzoate, chlorobenzoate, methylbenzoate, hydroxybenzoate, methoxybenzoate, phthalate, xylenesulphonate, phenylacetate, phenylpropionate, phenylbutyrate, citrate, lactate, gamma-hydroxybutyrate, glycolate, tartrate, methanesulphonate, propanesulphonate, naphthalene-1-sulfonate, napththalene-2-sulfonate, mandelate and the like. Preferred pharmaceutically acceptable acid addition salts are those formed with mineral acids such as hydrochloric acid and hydrobromic acid, and those formed with organic acids such as maleic acid and methanesulphonic acid.

Salts of amine groups may also comprise quarternary ammonium salts in which the amino nitrogen carries a suitable organic group such as an alkyl, alkenyl, alkynyl, or aralkyl moiety.

Base addition salts include those derived from inorganic bases, such as ammonium or alkali or alkaline earth metal hydroxides, carbonates, bicarbonates, and the like. Bases useful in preparing the salts of this invention thus include sodium hydroxide, potassium hydroxide, ammonium hydroxide, potassium carbonate, sodium carbonate, sodium bicarbonate, potassium bicarbonate, calcium hydroxide, calcium carbonate, and the like.

One skilled in the art will understand that the particular counterion forming a part of a salt of this invention is usually not of a critical nature, so long as the salt as a whole is pharmacologically acceptable and as long as the counterion does not contribute undesired qualities to the salt as a whole. The present invention further encompasses the pharmaceutically acceptable solvates of a compound of Formula I. Many of the compounds of Formula I can combine with solvents such as water, methanol, ethanol and acetonitrile to form pharmaceutically acceptable solvates such as the corresponding hydrate, methanolate, ethanolate and acetonitrilate.

The compounds of the present invention may have multiple asymmetric (chiral) centres. As a consequence of these chiral centres, the compounds of the present invention occur as racemates, mixtures of enantiomers and as individual enantiomers, as well as diastereomers and mixtures of diastereomers. All asymmetric forms, individual isomers and combinations thereof, are within the scope of the present invention.

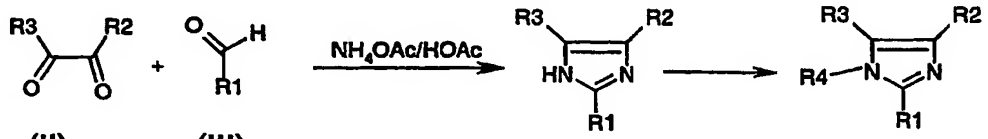
It will be readily understood by one skilled in the art that if the stereochemistry of a compound of Formula I is critical to its activity, then the relative stereochemistry of the compound is established early during synthesis to avoid subsequent stereoisomer separation problems. Further manipulation of the molecule will then employ stereospecific procedures so as to maintain the desired chirality.

Non-toxic metabolically-labile esters or amides of a compound of Formula I are those that are hydrolysed *in vivo* to afford the compound of Formula I and a pharmaceutically acceptable alcohol or amine. Examples of metabolically-labile esters include esters formed with (1-6C) alkanols, in which the alkanol moiety may be optionally substituted by a (1-8C) alkoxy group, for example methanol, ethanol, propanol and methoxyethanol. Examples of metabolically-labile amides include amides formed with amines such as methylamine.

II. Preparation of Compounds of Formula I

As is known in the art, triaryl imidazole compounds can be prepared by a number of standard techniques. Compounds of Formula I, therefore, can be prepared by several general synthetic methods, for example, as described by Grimmett, (Grimmett, M.R., *Comprehensive Heterocyclic Chemistry: The Structure, Reaction, Synthesis and Uses of Heterocyclic Compounds*, A. R. Katrizky and C. W. Rees, eds., Vol. 5, Pergamon Press. Oxford, 1984, pp. 457-498; Grimmett, M. R., *Imidazole and Benzimidazole Synthesis*, Academic Press, San Diego CA, 1997).

In one embodiment of the present invention, compounds of Formula I are prepared *via* solution or solid phase synthesis, by reacting a dione of Formula II with the aldehyde (III) at elevated temperature in the presence of ammonium acetate in acetic acid (see, for example, Krieg *et al., Naturforsch.* 1967, 22b:132; Sarshar *et al., Tetrahedron Lett.* 1996, 37:835-838).

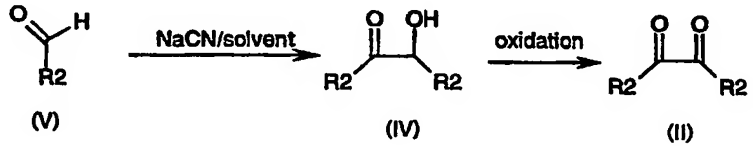


(II) (III)

The compounds of Formula (II) and (III) are either commercially available or may be prepared using standard procedures known to a person skilled in the relevant art.

Compounds of formula II can also be prepared:

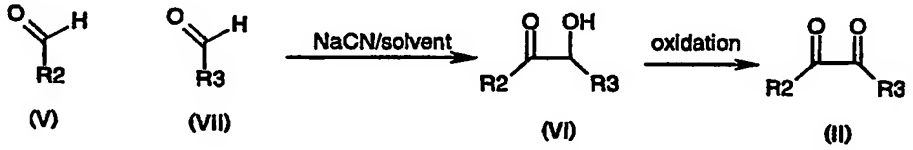
i) by oxidizing a compound of formula (IV). Compounds of formula (IV), in turn can be prepared by reacting a compounds of formula (V) with sodium cyanide in the presence of a solvent as shown below, wherein R2 is as defined above:



(V) (IV) (II)

or,

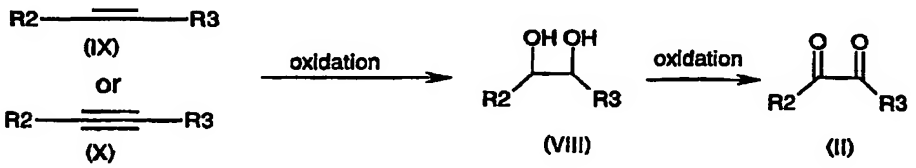
ii) by oxidizing a compound of formula (VI). Compounds of formula (VI), in turn can be prepared by treating a compound of formula (V) and a compound of formula (VII) with sodium cyanide in the presence of a solvent as shown below, wherein R2 and R3 are as defined above:



(V) (VII) (VI) (II)

or,

iii) by oxidizing a compound of formula (VIII). Compounds of formula (VIII) in turn can be prepared by oxidizing a compound of formula (IX) or (X) as shown below, wherein R2 and R3 are as defined above:



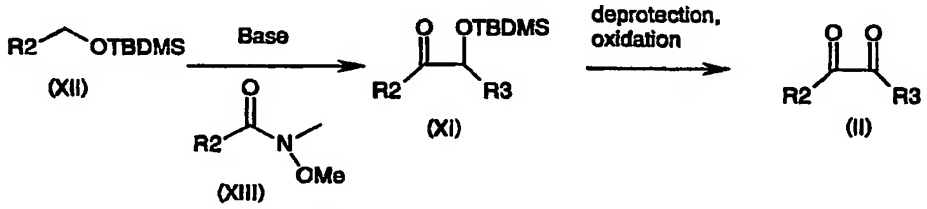
(VIII) (II)

or,

iv) by oxidizing a compound of formula (X) using $PdCl_2$ in DMSO,

or,

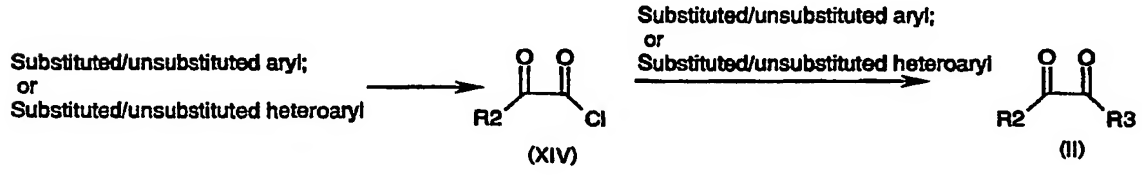
v) by deprotecting and oxidizing a compound of formula (XI). Compounds of formula (XI) in turn can be prepared by reacting a compound of formula (XII) with a compound of formula (XIII) in the presence of a suitable base:



wherein R2 and R3 are independently aryl, substituted aryl, heteroaryl or substituted heteroaryl,

or,

vi) by reacting a compound of formula (XIV) with a substituted or unsubstituted aryl or substituted or unsubstituted heteroaryl under Friedel Crafts acylation conditions or by nuecleophilic displacement of the chloride in compound of formula XIV. Compounds of formula (XIV) in turn can be prepared by reacting a substituted or unsubstituted aryl or substituted or unsubstituted heteroaryl with oxalyl chloride under Friedel Crafts acylation conditions:



wherein R2 and R3 are independently aryl, substituted aryl, heteroaryl or substituted heteroaryl.

III. Anti-Microbial Activity of Compounds of Formula I

The antimicrobial activity of a candidate compound of Formula I can be tested using standard techniques known in the art. In accordance with the present invention, the anti-microbial activity of a candidate compound may be anti-bacterial activity or it may be anti-fungal activity, or the compound may exhibit both anti-bacterial and anti-fungal activity. As is known in the art, anti-microbial activity of the compounds may result in the killing of microbial cells (*i.e.* bacteriocidal and/or fungicidal activity), or it may result in the slowing or arrest of the growth of microbial cells (*i.e.* bacteriostatic or fungistatic activity). Thus the compounds of Formula I may be bacteriocidal and/or fungicidal or they may be bacteriostatic and/or fungistatic. Compounds of the present invention that slow or arrest microbial cell growth may be useful in combination treatments with other known anti-microbial agents.

A. In vitro *Testing*

In vitro methods of determining the ability of candidate compounds to inhibit, prevent or eradicate the growth of microbial cells are well-known in the art. In general, these methods involve contacting a culture of the cells of interest with various concentrations of the candidate compound and monitoring the growth of the cell culture relative to a control culture not contacted with the compound. A second control culture that comprises cells contacted with a known anti-microbial agent may also be included.

For example, the ability of a candidate compound of Formula I to inhibit the growth of microbial cells can readily be determined by measurement of the minimum inhibitory concentration (MIC) for the compound. The MIC is defined as the lowest concentration that inhibits growth of the organism to a pre-determined extent. For example, a MIC_{100} value is

defined as the lowest concentration that completely inhibits growth of the organism, whereas a MIC_{90} value is defined as the lowest concentration that inhibits growth by 90% and a MIC_{50} value is defined as the lowest concentration that inhibits growth by 50%. MIC values are sometimes expressed as ranges, for example, the MIC_{100} for a compound may be expressed as the concentration at which no growth is observed or as a range between the concentration at which no growth is observed and the concentration of the dilution which immediately follows.

Typically, MICs for candidate anti-bacterial compounds are measured using a broth macro- or microdilution assay (see Amsterdam, D. (1996) "Susceptibility testing of antimicrobials in liquid media," pp.52-111. In Loman, V., ed. *Antibiotics in Laboratory Medicine*, 4th ed. Williams and Wilkins, Baltimore, MD). In the classical broth microdilution method, the candidate anti-bacterial compound is diluted in culture medium in a sterile, covered 96-well microtiter plate. Culture medium only (containing no bacteria) is also included as a negative control for each plate and known antibiotics are often included as positive controls. An overnight culture of a single bacterial colony is diluted in sterile medium such that, after inoculation, each well in the microtiter plate contains an appropriate number of colony forming units (CFU)/ml (typically approximately 5×10^5 CFU/ml). The inoculated microtiter plate is subsequently incubated at an appropriate temperature (for example, 35 C – 37 C for 16-48 hours. The turbidity of each well is then determined by visual inspection and/or by measuring the absorbance, or optical density (OD), at 595nm or 600nm using a microplate reader and is used as an indication of the extent of bacterial growth.

Techniques for determining MIC values for candidate anti-fungal compounds are similar to those outlined above for anti-bacterial compounds and include both macrodilution and microdilution methods (see, for example, Pfaller, M.A., Rex, J.H., Rinaldi, M.G., *Clin. Infect. Dis.*, (1997) 24:776-84). A standardised anti-fungal susceptibility test method, NCCLS M27-T, has been proposed by the National Committee for Clinical Laboratory Standards (NCCLS) (see, Ghannoum, M.A., Rex, J.H. and Galgiani J.N., *J. Clin. Microbiol.*, (1996) 34:489-495; Pfaller, M.A. and Yu, W.L., *Infect. Dis. Clin. North Amer.*, (2001) 15:1227-1261).

In accordance with the present invention, a compound of Formula I is considered to have an anti-microbial effect against a given micro-organism when the MIC for complete inhibition of growth of the organism is less than about 75 μg/ml. In one embodiment, the compound has a MIC less than about 50 μg/ml for the relevant micro-organism. In related embodiments, the compound has a MIC of less than about 25 μg/ml and less than about 12.5 μg/ml for the relevant micro-organism.

As is known in the art, many anti-microbial compounds show maximal effects when used in combination with a second drug. Such effects can be simply additive, or they can be synergistic. For example, a compound that exhibits only bacteriostatic effects when used in isolation can become bacteriocidal when used in combination with a second anti-bacterial compound. Thus, the present invention contemplates that the anti-microbial activity of a compound of Formula I may be synergistically enhanced by the presence of another compound of Formula I, or by the presence of another known anti-microbial agent. Methods of testing for synergistic effects between two or more compounds are well-known in the art.

For example, the fractional inhibitory concentration (FIC) can be used to determine synergy between two anti-bacterial compounds (see, for example, U.S. Patent No. 6,288,212). FICs are determined in microtiter plates in a similar manner to MICs, except that FICs are performed using a checkerboard titration of, for example, candidate compounds in one dimension and known antibiotics in the other dimension. The FIC is calculated by evaluating the impact of one antibiotic on the MIC of the other and vice versa. As used herein, FIC can be determined as follows:

$$FIC = \frac{\text{MIC (}\textit{candidate compound in combination}\text{)}}{\text{MIC (}\textit{candidate compound alone}\text{)}} + \frac{\text{MIC (}\textit{known antibiotic in combination}\text{)}}{\text{MIC (}\textit{known antibiotic alone}\text{)}}$$

An FIC value equal to one indicates that the influence of the compounds is additive and an FIC value of less than one indicates synergy. An FIC value of less than 0.5 is typically obtained for synergism.

B. In vivo *Testing*

The ability of a compound of Formula I to act as an anti-microbial agent can also be tested *in vivo* using standard techniques. A number of animal models are known in the art that are suitable for testing the activity of anti-microbial compounds and are readily available.

Representative examples of animal models suitable for testing the anti-bacterial activity of a compound of Formula I *in vivo* include, but are not limited to, the immunosuppressed mouse as a model of acute *Staphylococcus aureus* infection, the burnt mouse or neutropenic mouse as a model for *Pseudomonas aeruginosa* infections and the suckling mouse for *Vibrio cholerae* infection of the intestine (Klose, *et al.,* (2000), *Trends Microbiol.,* 8:189-91).

Other examples of suitable models and procedures for *in vivo* testing of anti-bacterial compounds are described in Iwahi, T., *et al., J. Med. Microbiol.,* (1982) 15:303-316; Michie, H.R., *J. Antimicrob. Chemother.,* (1998) 41:47-49; Yanke, S.J., *et al., Can. J. Microbiol.,* (2000) 46:920-926; Shibata, K., *et al., J. Antimicrob. Chemother.,* (2000) 45:379-82; Totsuka, K., *et al., J. Antimicrob. Chemother.,* (1999) 44:455-60; Goto, Y., *et al., Int. J. Antimicrob. Agents.,* (1999) 11:39-46.

Representative examples of animal models suitable for testing the anti-fungal activity of a compound of Formula I *in vivo* include, but are not limited to, the severe combined immunodeficiency (SCID) mouse model and a colostrum-deprived SPF piglet model for *Cryptosporidium parvum* infection, a granulocytopenic rabbit model of disseminated Candidiasis (see, for example, Walsh, *et al., J. Infect. Dis.,* 1990, 161:755-760; Thaler, *et al., J. Infect. Dis.,* 1988, 158:80), a mouse model of disseminated Aspergillosis (see, for example, Arroyo, *et al., Antimicrob. Agents Chemoth.,* 1977, pp. 21-25) and a neutropenic

rat model of disseminated Candidiasis (see, for example, Lechner, *et al., Am. J. Physiol. (Lung Cell. Mol. Physiol.)* 1994, 10:1-8).

Methods for conducting *in vivo* tests to determine the activity of antimicrobial compounds are well-known in the art. Typically, *in vivo* testing comprises introducing a selected micro-organism into the appropriate animal model in a sufficient amount to cause infection, followed by administration of one or more doses of the test compound of Formula I. Administration is generally by bolus infusion into a suitable vein (for example the tail vein of mice or rats). Animals treated with a known antimicrobial agent and/or with a saline or buffer control solution serve as controls. Repeat doses of the test compound may be administered to the animal, if necessary, at appropriate time intervals. The animals are subsequently monitored daily for mortality.

In accordance with the present invention, a compound of Formula I is considered to exert an *in vivo* anti-microbial effect if it results in a decrease in mortality of at least about 15% in a treated animal compared to a test animal. In one embodiment of the present invention, the compound of Formula I results in a decrease in mortality of at least about 25% in the treated animal. In a related embodiment, the compound results in a decrease in mortality of at least about 40%. In other related embodiments, the compound results in a decrease in mortality of at least about 50%, 60%, 70%, 80% and 90% in the treated animal.

IV. Toxicity Testing

It is important that the anti-microbial compounds of the present invention exhibit low toxicity *in vivo*. Toxicity tests for potential drugs are well-known in the art (see, for example, Hayes, A.W., ed., (1994), *Principles and Methods of Toxicology,* 3rd ed., Raven Press, NY; Maines, M., ed., *Current Protocols in Toxicology,* John Wiley & Sons, Inc., NY).

In vitro acute toxicity testing of a compound of Formula I can be performed using mammalian cell lines (see, for example, Ekwall, B., *Ann. N.Y. Acad. Sci.,* (1983) 407:64-77).

Selection of an appropriate cell line is dependent on the potential application of the candidate compound and can be readily determined by one skilled in the art.

In vivo toxicity testing can be performed by standard methodology. For example, by injecting varying concentrations of the candidate compound into an appropriate animal model. The compound can be injected once, or administration can be repeated over several days. The toxic effects of the compound can be evaluated over an appropriate time period by monitoring the general health and body weight of the animals. After the completion of the period of assessment, the animals can be sacrificed and the appearance and weight of the relevant organs determined.

In accordance with the present invention, a compound of Formula I for use *in vivo* shows both good anti-microbial activity and low or no toxicity at the concentration at which it would be administered as an anti-microbial agent.

V. Uses of the Anti-Microbial Compounds of Formula I

The present invention provides for the use of one or more compounds of Formula I for the inhibition, prevention or eradication of the growth or proliferation of bacteria and/or fungi, either alone or in combination with one or more other compounds of Formula I or known anti-microbial agents.

Thus, in one embodiment, the present invention provides a method of inhibiting bacterial growth by contacting a bacterium with an effective amount of one or more compounds of Formula I. The compounds of Formula I may have broad spectrum anti-bacterial activity, in which case they may be used against gram-positive or gram-negative bacteria. Representative examples of bacteria that may be inhibited by compounds of Formula I include, but are not limited to, *Corynebacterium xerosis, Chlamydia pneumoniae, Chlamydia trachomatis, Enterobacter cloacae, Enterobacter facaelis, Enterococcus faecium, Escherichia coli, Escherichia coli O157:H7, Haemophilus influenzae, Helicobacter pylori, Listeria monocytogenes, Moraxella catarrhalis, Neisseria gonorrhoae, Neisseria meningitidis, Pseudomonas aeruginosa,* Pneumococci species, *Salmonella enterica,*

Salmonella typhimurium, Staphylococcus aureus, Staphylococcus aureus K147, Staphylococcus epidermidis, Staphylococcus typhimurium, Streptococcus mitis, Streptococcus pneumoniae, Streptococcus pyogenes, Vibrio cholerae, Mycobacterium tuberculosis and other acid-fast staining bacteria (*i.e. M. africanum, M. avium-intracellulare, M. pneumoniae, M. bovis, M. leprae, M. phlei*), *Bacillus anthracis* and other endospore-forming rods and cocci.

It is well-established in the field of microbiology that many multidrug-resistant strains of bacteria have emerged in the recent past and will continue to emerge with the continued use of standard antibiotics. Examples of currently known resistant strains of bacteria include methicillin-resistant *Staphylococcus aureus* (MRSA), methicillin-resistant coagulase-negative staphylococci (MRCNS), penicillin-resistant *Streptococcus pneumoniae,* penicillin-resistant pneumococci and multidrug-resistant *Enterococcus faecium*. The present invention, therefore, contemplates the use of compounds of Formula I in the inhibition of growth of such multidrug-resistant strains. In one embodiment of the present invention, compounds of Formula I are used to inhibit the growth of MRSA.

In accordance with the present invention, one or more anti-bacterial compounds of Formula I can be administered in a therapeutically effective amount alone or in combination with one or more other anti-bacterial agents to a subject with a bacterial disorder. Thus, the present invention provides the use of one or more of the compounds of Formula I in the treatment of bacterial infections and bacterially-related disorders and diseases. Examples of bacterially-related disorders and diseases that may be treated with the compounds of the present invention include, but are not limited to, tuberculosis, meningitis, ulcers, septicaemia, bacteremia, cystic fibrosis, pneumonia, typhoid fever, bacterial conjunctivitis, gonorrhoea, impetigo, bacterial eye or ear infections, bacterial diarrhoea, cystitis, bacterial vaginitis, bacterial endocarditis, bacterial pericarditis, peliosis, superficial skin infections, toxic shock, food poisoning, hemolytic uremic syndrome, botulism, leprosy, gangrene, tetanus, lyme disease, plague, anthrax and chancroid.

In another embodiment, the present invention provides a method of inhibiting fungal growth by contacting a fungus with an effective amount of one or more compounds of Formula I either alone or in combination with one or more other anti-fungal agents. Representative examples of fungi that may be inhibited with compounds of Formula I include, but are not limited to, Histoplasma (*e.g. H. capsulstum*), Coccidioides, Blastomyces, Paracoccidioides, Cryptococcus (*e.g. C. neoformans*), Aspergillus (*e.g. A. fumigatus, A. flaws, A. niger, A. nidulans, A. terreus, A. sydowi, A. flavatus,* and *A. glaucus*), Zygomycetes (*e.g.* Basidiobolus, Conidiobolus, Rhizopus, Mucor, Absidia, Mortierella, Cunninghamella, and Saksenaea), Candida (*e.g. C. albicans, C. tropicalis, C. parapsilosis, C. stellatoidea, C. krusei, C. parakrusei, C lusitaniae, C. pseudorropicalis, C. guilliermondi* and *C. glabrata*), *Cryptosporidium parvum, Sporothrix schenckii, Piedraia hortae, Trichosporon beigelii, Malassezia furfur, Phialophora verrucosa, Fonsecae pedrosoi, Madurella mycetomatis* and *Pneumocystis carinii.*

In accordance with the present invention, one or more anti-microbial compounds of Formula I can be administered in a therapeutically effective amount either alone or in combination with other anti-fungal agents to a subject with a fungal infection or fungally-related disorder or disease. Examples of fungally-related disorders and diseases that may be treated with the compounds of Formula I include, but are not limited to, Candidiasis; endemic mycoses (such as Histoplasmosis, Coccidioidomycosis, Blastomycosis, Paracoccidioidomycosis, Cryptococcosis, Aspergillosis, Mucormycosis), associated disseminated infections and progressive pulmonary disease; cryptococcal meningitis; narcotising patchy bronchopneumonia; haemorrhagic pulmonary infarction; rhinocerebral disease; neutropenia, black piedra; white piedra; tinea (versicolor, capitis, corporis, *etc.*); *Pneumocystis* pneumonia; chromoblastomycosis, and maduromycosis.

In accordance with the present invention, one or more compounds of Formula I may be used as therapeutic agents for the treatment of infection, disorders or diseases, in combination with one or more known drugs in combination or synergistic therapy. Such therapy is known in the art and selection of the appropriate drug(s) to be administered with a compound of Formula I is easily discernible by one of skill in the art. For example, in the treatment of

bacterial infections and related diseases, useful classes of antibiotics for combination or synergistic therapy include, but are not limited to, aminoglycosides, penicillins, cephalosporins, fluoroquinolones, quinolones, carbapenems, tetracyclines, glycopeptides and macrolides, and other antibiotics such as chloramphenicol, clindamycin, trimethoprim, sulphamethoxazole, nitrofurantoin, rifampin and mupirocin. For the treatment of fungal infections and fungally-related diseases, candidate antimicrobial compounds for combination therapy include, but are not limited to, amphotericin B and the structurally related compounds nystatin and pimaricin; flucytosine; azole derivatives such as ketoconazole, clotrimazole, miconazole, econazole, butoconazole, oxiconazole, sulconazole, terconazole, fluconazole and itraconazole; allylamines-thiocarbamates, such as tolnaftate and naftifme, and griseofulvin.

The present invention also contemplates the use of compounds of Formula I as the active ingredient in anti-microbial cleansers, polishes, paints, sprays, soaps, or detergents. The compounds may be formulated for application to surfaces to inhibit the growth of a microbial species thereon, for example, surfaces such as countertops, desks, chairs, laboratory benches, tables, floors, sinks, showers, toilets, bathtubs, bed stands, tools or equipment, doorknobs and windows. Alternatively, the compounds may be formulated for laundry applications, for example, for washing clothes, towels, sheets and other bedlinen, washcloths or other cleaning articles. The antimicrobial cleansers, polishes, paints, sprays, soaps, or detergents according to the present invention can optionally contain suitable solvent(s), carrier(s), thickeners, pigments, fragrances, deodorisers, emulsifiers, surfactants, wetting agents, waxes, or oils. In one embodiment, the present invention provides a formulation containing one or more compounds of Formula I for external use as a pharmaceutically acceptable skin cleanser. The cleansers, polishes, paints, sprays, soaps, and detergents according to the present invention are useful institutions, such as in hospital settings for the prevention of nosocomial infections, as well as in home settings.

In addition, the invention contemplates the use of compounds of Formula I in formulations to kill or inhibit the growth of microbial species in food preparations, or to sterilise surgical and other medical equipment and implantable devices, including prosthetic joints. The

compounds can also be formulated for use in the *in situ* sterilisation of indwelling invasive devices such as intravenous lines and catheters, which are often foci of infection.

The present invention further contemplates the use of the compounds of Formula I as the active ingredient in personal care items, such as soaps, deodorants, shampoos, mouthwashes, toothpastes, and the like. Many compositions used in personal care applications are susceptible to microbial growth and it is thus desirable to incorporate into these compositions an effective anti-microbial material. The anti-microbial agent may be incorporated into the personal care formulation using techniques known in the art. Thus, the anti-microbial agent may be added to the personal care formulation as a solution, emulsion or dispersion in a suitable liquid medium. Alternatively, the anti-microbial agent may be added, undiluted, to the personal care formulation or may be added with a solid carrier or diluent. The anti-microbial agent may be added to the pre-formed personal care formulation or may be added during the formation of the personal care formulation, either separately or premixed with one of the other components of the formulation.

VI. Pharmaceutical Formulations and Administration of Anti-Microbial Compounds of Formula I

For use as therapeutic agents in the treatment of infections, disorders or disease in a subject, the anti-microbial compounds of the present invention are typically formulated prior to administration. Therefore, the present invention provides pharmaceutical formulations comprising one or more compounds of Formula I and a pharmaceutically-acceptable carrier, diluent, or excipient. The present pharmaceutical formulations are prepared by standard procedures using well-known and readily available ingredients. In making the compositions of the present invention, the active ingredient will usually be mixed with a carrier, or diluted by a carrier, or enclosed within a carrier, and may be in the form of a capsule, sachet, paper, or other container. When the carrier serves as a diluent, it may be a solid, semi-solid, or liquid material that acts as a vehicle, excipient, or medium for the active ingredient.

The pharmaceutical compositions comprising the anti-microbial compounds according to the present invention may be administered in a number of ways depending upon whether local or systemic treatment is desired and upon the area to be treated. Administration may be topical (including ophthalmic and to mucous membranes including vaginal and rectal delivery), pulmonary, *e.g.* by inhalation or insufflation of powders or aerosols, including by nebulizer; intratracheal, intranasal, epidermal and transdermal, oral or parenteral. Parenteral administration includes intravenous, intraarterial, subcutaneous, intraperitoneal or intramuscular injection or infusion; or intracranial, *e.g.* intrathecal or intraventricular, administration.

The anti-microbial compounds of the present invention may be delivered alone or in combination, and may be delivered along with a pharmaceutically acceptable vehicle. Ideally, such a vehicle would enhance the stability and/or delivery properties. The present invention also provides for administration of pharmaceutical compositions comprising one or more of the compounds of Formula I using a suitable vehicle, such as a liposome, microparticle or microcapsule. The use of such vehicles may be beneficial in achieving sustained release of the anti-microbial compound(s).

For administration to an individual for the treatment of an infection or disease, the present invention also contemplates the formulation of the pharmaceutical compositions comprising the anti-microbial compounds into oral dosage forms such as tablets, capsules and the like. For this purpose, the compounds can be combined with conventional carriers, such as magnesium carbonate, magnesium stearate, talc, sugar, lactose, pectin, dextrin, starch, gelatine, tragacanth, methylcellulose, sodium carboxymethyl-cellulose, low melting wax, cocoa butter and the like. Diluents, flavouring agents, solubilizers, lubricants, suspending agents, binders, tablet-disintegrating agents and the like can also be employed, if required. The anti-microbial compounds can be encapsulated with or without other carriers. In accordance with the present invention, the proportion of anti-microbial compound(s) in any solid and liquid composition will be at least sufficient to impart the desired activity to the individual being treated upon oral administration. The present invention further contemplates parenteral injection of the anti-microbial compounds, in which case the

compounds are formulated as a sterile solution containing other solutes, for example, enough saline or glucose to make the solution isotonic.

For administration by inhalation or insufflation, the anti-microbial compounds can be formulated into an aqueous or partially aqueous solution, which can then be utilized in the form of an aerosol. Aqueous formulations of the anti-microbial compounds of the present invention may also be used in the form of ear or eye drops, or ophthalmic solutions. The present invention further contemplates topical use of the anti-microbial compounds. For this purpose they can be formulated as dusting powders, creams or lotions in pharmaceutically acceptable vehicles, which are applied to affected portions of the skin.

Compositions intended for oral use may be prepared according to procedures known in the art for the manufacture of pharmaceutical compositions and such compositions may further contain one or more sweetening agents, flavouring agents, colouring agents, preserving agents, or a combination thereof, in order to provide pharmaceutically elegant and palatable preparations. Tablets typically contain the anti-microbial compound(s) in admixture with non-toxic pharmaceutically acceptable excipients suitable for the manufacture of tablets, such as inert diluents, for example, calcium carbonate, sodium carbonate, lactose, calcium phosphate or sodium phosphate; granulating and disintegrating agents, for example, corn starch, or alginic acid; binding agents, for example, starch, gelatine or acacia, and lubricating agents, for example, magnesium stearate, stearic acid or talc. The tablets may be uncoated or they may be coated by known techniques to delay disintegration and absorption in the gastrointestinal tract and thereby provide a sustained action over a longer period. For example, a time delay material such as glyceryl monostearate or glyceryl distearate may be employed.

Formulations for oral use may also be presented as hard gelatine capsules wherein the anti-microbial compound(s) is mixed with an inert solid diluent, for example, calcium carbonate, calcium phosphate or kaolin, or as soft gelatine capsules wherein the active ingredient is mixed with water or an oil medium, for example, peanut oil, liquid paraffin or olive oil.

Aqueous suspensions typically contain the anti-microbial compound(s) in admixture with excipients suitable for the manufacture of aqueous suspensions, such as suspending agents (for example, sodium carboxylmethylcellulose, methyl cellulose, hydropropylmethylcellulose, sodium alginate, polyvinylpyrrolidone, gum tragacanth and gum acacia); dispersing or wetting agents such as a naturally-occurring phosphatide (for example, lecithin), or condensation products of an alkylene oxide with fatty acids (for example, polyoxyethylene stearate), or condensation products of ethylene oxide with long chain aliphatic alcohols (for example, hepta-decaethyleneoxycetanol), or condensation products of ethylene oxide with partial esters derived from fatty acids and a hexitol (for example, polyoxyethylene sorbitol monooleate), or condensation products of ethylene oxide with partial esters derived from fatty acids and hexitol anhydrides (for example, polyethylene sorbitan monooleate). The aqueous suspensions may further contain one or more preservatives, for example, ethyl, or *n*-propyl-*p*-hydroxy benzoate; one or more colouring agents; one or more flavouring agents, or one or more sweetening agents, such as sucrose or saccharin, or a combination thereof.

Oily suspensions may be formulated by suspending the anti-microbial compound(s) in a vegetable oil, for example, peanut oil, olive oil, sesame oil or coconut oil, or in a mineral oil such as liquid paraffin. The oily suspensions may contain a thickening agent, for example beeswax, hard paraffin or cetyl alcohol. Sweetening agents such as those set forth above, and flavouring agents may be added to provide palatable oral preparations. These compositions may be preserved by the addition of an anti-oxidant such as ascorbic acid.

Dispersible powders and granules suitable for preparation of an aqueous suspension by the addition of water provide the anti-microbial compound in admixture with a dispersing or wetting agent, suspending agent and one or more preservatives. Suitable dispersing or wetting agents and suspending agents are exemplified by those mentioned above. Additional excipients, for example, sweetening, flavouring and colouring agents, may also be present.

Pharmaceutical compositions of the present invention may also be in the form of oil-in-water emulsions. The oil phase may be a vegetable oil, for example, olive oil or peanut oil, or a mineral oil, for example, liquid paraffin, or mixtures thereof. Suitable emulsifying agents may be naturally-occurring gums (for example, gum acacia or gum tragacanth); naturally-occurring phosphatides (for example, soy bean lecithin), and esters or partial esters derived from fatty acids and hexitol anhydrides (for example, sorbitan monooleate), and condensation products of the partial esters with ethylene oxide (for example, polyoxyethylene sorbitan monooleate). The emulsions may also contain sweetening and flavouring agents.

Syrups and elixirs may be formulated with sweetening agents, for example, glycerol, propylene glycol, sorbitol or sucrose. Such formulations may also contain one or more demulcents, preservatives or flavouring and colouring agents, or combinations thereof.

The pharmaceutical compositions may be in the form of a sterile injectable aqueous or oleaginous suspension. This suspension may be formulated according to known art using suitable dispersing or wetting agents and suspending agents as described above. The sterile injectable preparation may also be a solution or a suspension in a non-toxic, parentally acceptable diluent or solvent, for example, as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. Typically, a bland fixed oil is employed for this purpose such as a synthetic mono- or diglyceride. In addition, fatty acids such as oleic acid find use in the preparation of injectables. Adjuvants, such as local anaesthetics, preservatives and buffering agents, may also be included in the injectable formulation.

The compound(s) of Formula I may be administered, together or separately, in the form of suppositories for rectal or vaginal administration of the compound. These compositions can be prepared by mixing the compound with a suitable non-irritating excipient which is solid at ordinary temperatures but liquid at the rectal/vaginal temperature and will therefore melt

to release the compound. Examples of such materials include cocoa butter and polyethylene glycols.

Another formulation of the present invention employs transdermal delivery devices ("patches"). Such transdermal patches may be used to provide continuous or discontinuous infusion of the anti-microbial compounds of the present invention in controlled amounts. The construction and use of transdermal patches for the delivery of pharmaceutical agents is well known in the art (see, for example, U.S. Patent No. 5,023,252; issued Jun. 11, 1991) herein incorporated by reference. Such patches may be constructed for continuous, pulsatile, or on demand delivery of pharmaceutical agents.

It may be desirable or necessary to introduce the pharmaceutical composition to the brain, either directly or indirectly. Direct techniques usually involve placement of a drug delivery catheter into the host's ventricular system to bypass the blood-brain barrier. An example of such an implantable delivery system, used for the transport of biological factors to specific anatomical regions of the body, is described in U.S. Patent No. 5,011,472.

The dosage of the anti-microbial compound to be administered is not subject to defined limits, but will usually be an effective amount. In general, the dosage will be the equivalent, on a molar basis, of the pharmacologically active free form produced from a dosage formulation upon the metabolic release of the active free drug to achieve its desired pharmacological and physiological effects. The pharmaceutical compositions are typically formulated in a unit dosage form, each dosage containing from about 0.05 to about 100 mg, more usually about 1.0 to about 30 mg, of the anti-microbial compound. The term "unit dosage form" refers to physically discrete units suitable as unitary dosages for human subjects and other mammals, each unit containing a predetermined quantity of anti-microbial compound calculated to produce the desired therapeutic effect, in association with a suitable pharmaceutical excipient.

Typical daily dosages of the anti-microbial compounds fall within the range of about 0.01 to about 100 mg/kg of body weight. Usually, daily doses will be about 0.05 mg/kg to about 50

mg/kg, more usually from about 0.1 mg/kg to about 25 mg/kg. In the treatment of adult humans, the dose may be in the range of about 0.1 to about 15 mg/kg/day, in single or divided dose. However, it will be understood that the amount of the compound actually administered will be determined by a physician, in the light of the relevant circumstances, including the condition to be treated, the chosen route of administration, the actual compound administered, the age, weight, and response of the individual patient, and the severity of the patient's symptoms, and therefore the above dosage ranges are not intended to limit the scope of the invention in any way. In some instances dosage levels below the lower limit of the aforesaid range may be more than adequate, while in other cases still larger doses may be employed without causing any harmful side effect, for example, by first dividing larger doses into several smaller doses for administration throughout the day.

VII. Kits

The present invention additionally provides for therapeutic kits containing one or more compounds of Formula I in pharmaceutical compositions for use in the treatment of infections and disease. Individual components of the kit would be packaged in separate containers and, associated with such containers, can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human or animal administration.

When the components of the kit are provided in one or more liquid solutions, the liquid solution can be an aqueous solution, for example a sterile aqueous solution. For *in vivo* use, the anti-microbial compound may be formulated into a pharmaceutically acceptable syringeable formulation. In this case the container means may itself be an inhalant, syringe, pipette, eye dropper, or other such like apparatus, from which the formulation may be applied to an infected area of the animal, such as the lungs, injected into an animal, or even applied to and mixed with the other components of the kit.

The components of the kit may also be provided in dried or lyophilised forms. When reagents or components are provided as a dried form, reconstitution generally is by the addition of a suitable solvent. It is envisioned that the solvent also may be provided in another container means. Irrespective of the number or type of containers, the kits of the invention also may comprise, or be packaged with, an instrument for assisting with the injection/administration or placement of the ultimate composition within the body of an animal. Such an instrument may be an inhalant, syringe, pipette, forceps, measured spoon, eye dropper or any such medically approved delivery vehicle.

To gain a better understanding of the invention described herein, the following examples are set forth. It should be understood that these examples are for illustrative purposes only. Therefore they should not limit the scope of the invention in any way.

EXAMPLES

EXAMPLE 1: **In vitro *Inhibition of Methicillin-Resistant* Staphylococcus aureus *(MRSA)***

CMRSA-1B is an epidemic multi-drug resistant strain of *S. aureus* which accounts for 49% and 70% of *S. aureus* strains isolated in hospitals in Canada and in Ontario respectively. CMRSA-1B was cultured in Tryptic Soy Broth (TSB) at 37°C and used for the inhibition assays during the log phase of growth (OD_{600} of 0.1). 10µl of each candidate compound were placed in duplicate into the wells of a 96-microtitre plate followed by the addition of 90 µl of the CMRSA-1B culture suspensions. The candidate compounds were dissolved at a concentration of 250 µg/ml in 50 % DMSO, and diluted to a final concentration of 25 µg/ml in 5 % DMSO in the culture suspension. Bacterial growth was monitored by measuring the absorbance at 600 nm in an ELISA reader. The level of growth inhibition was estimated as percentage of the OD_{600} value with respect to a control consisting of an aliquot of the same bacterial suspension in the presence of 5 % DMSO.

A. Determination of Minimal Inhibitory Concentration (MIC)

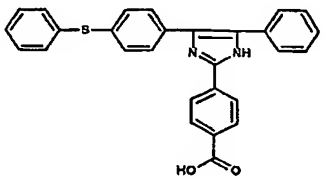
The lowest concentration of triaryl-imidazole derivative that completely inhibited growth of the micro-organisms *in vitro* (MIC) was determined by the sequential macrodilution (tube) broth method. (Nat. Committee for Clinical Laboratory Standards. *Document M7-A5* 2000, 20;1-25). Bacterial suspensions containing 5 x 10^5 colony-forming units (CFU) were incubated with serial two-fold dilutions of each drug at 37°C overnight, and growth was monitored visually. The range of MIC values among the triaryl-imidazole derivatives was 12.5 – 50 µg/ml. Table 1 shows the MIC value for some of the 2,4,5-triarylimidazole-derivatives against MRSA.

Table 1

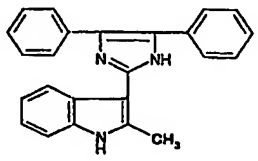
Conc. µg/ml	Compound ML-12	Compound ML-42	Compound ML-6	Compound ML-7
0	+	+	+	+
3.12	+	+	+	+
6.25	+	+	+	+
12.50	-	-	+	+
25.00	-	-	-	+
50.00	-	-	-	-
100.00	-	-	-	-

(+): Positive visual bacterial growth

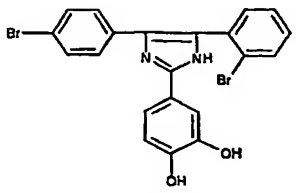
(-): Negative visual bacterial growth



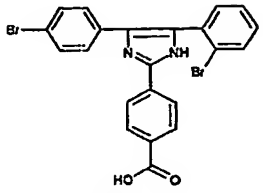
ML-12



ML-42



ML-6



ML-7

B. Bactericidal Effect against MRSA

The bactericidal effect of compounds of Formula I against MRSA was determined using the same concentrations of compounds and growth conditions used to determine the MICs (see above). Serial dilutions of liquid cultures were incubated with the compounds at 37°C overnight and then plated on Tryptic Soy Agar (TSA) plates. After incubation for 17 hours at 37°C, the number of live bacteria was calculated from the number of colonies grown per milliliter of culture and expressed as colony-forming units (CFU). A bactericidal effect was observed at the corresponding MIC for derivatives ML-12 and ML-42 while derivative ML-6 and ML-7 exhibited a bacteriostatic effect at these concentrations (Figure 1).

EXAMPLE 2: **In vivo *Inhibition of Methicillin-Resistant* Staphylococcus aureus *(MRSA)***

Triaryl-imidazole compounds were tested *in vivo* for antibacterial activity against MRSA in a model of acute infection using immunosupressed mice. Suspensions of 400 µL containing 5×10^6 MRSA-1B387 bacteria in 5 % mucin were injected into groups of 5-10 female, 8-14 weeks CB17-SCID mice. Under these conditions bacterial infection produced 80-100 % lethality in less than 48 hours. Each group of inoculated mice was treated with 50 mg/kg of the respective compound intraperitoneally (I.P.) immediately and 3 hours after bacterial inoculation. The results of the *in vivo* experiments are shown in Table 2.

Table 2

Compound	No. of Experiments	Survival (%)
ML-12	1	40
ML-42	1	80
ML-6	2	15
ML-7	3	50
Vancomycin	2	100
Control	4	24

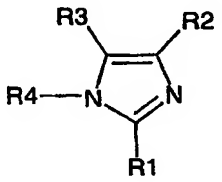
EXAMPLE 3: **In vivo *Toxicity Tests***

In vivo acute toxicity tests were conducted using compounds ML-12, ML-42, ML-6 and ML-7. Mice were injected with each of the above compounds at a concentration of 200 mg/kg per day. No symptoms of sickness, change of total weight, organ weight and appearance was observed in any of the mice challenged, indicating that these compounds exhibit no toxic effects in mice.

Further *in vivo* GLP toxicology studies are conducted using using different animal species.

THE EMBODIMENTS OF THE INVENTION IN WHICH AN EXCLUSIVE PROPERTY OR PRIVILEGE IS CLAIMED ARE DEFINED AS FOLLOWS:

1. A compound having structural formula (I):



(I)

wherein:

R1, R2 and R3 are independently aryl, substituted aryl, heterocycle, heteroaryl, substituted heterocycle, or substituted heteroaryl; and

R4 is hydrogen, halogen, hydroxyl, thiol, lower alkyl, substituted lower alkyl, alkenyl, alkenyl, alkylalkenyl, alkyl alkynyl, alkoxy, alkylthio, acyl, aryloxy, amino, amido, carboxyl, aryl, substituted aryl, heterocycle, heteroaryl, substituted heterocycle, heteroalkyl, cycloalkyl, substituted cycloalkyl, alkylcycloalkyl, alkylcycloheteroalkyl, nitro, or cyano.

2. Use of a compound of Formula I in the treatment of a bacterial or fungal infection in a mammal in need of such therapy.

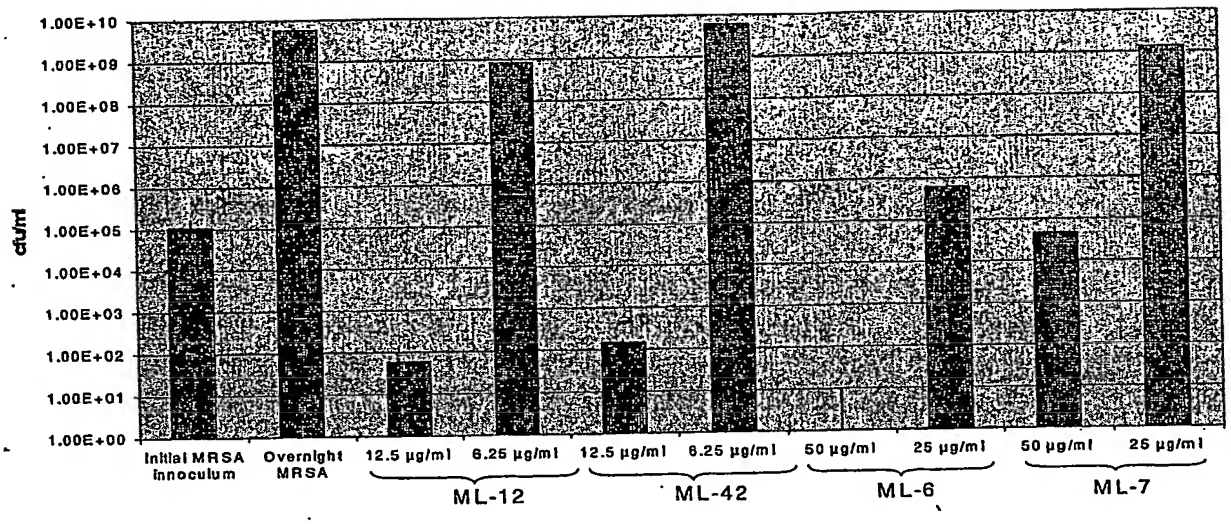


Figure 1: Bactericidal effect of some triaryl-imidazole derivatives against MRSA